

71531 U.S. PTO
05/06/97

A/NO FEE

PATENT
Atty. Docket No. CRP-145

74280 U.S. PTO
08851628
05/06/97

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of
Inventor(s): Kuber T. Sampath and Charles M. Cohen

WARNING: Patent must be applied for in the name(s) of all of the actual inventor(s). 37 CFR 1.41(a) and 1.53(b)

For (title): NOVEL THERAPIES FOR CHRONIC RENAL FAILURE

1. Type of Application

This new application is for a(n) (check one applicable item below):

- ☒ Original
☐ Design
☐ Plant

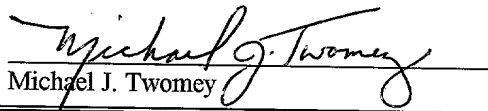
WARNING: Do not use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4) unless the International Application is being filed as a divisional, continuation or continuation-in-part application.

NOTE: If one of the following 3 items apply then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED.

- ☐ Divisional
☐ Continuation
☐ Continuation-in-Part

CERTIFICATE OF EXPRESS MAILING UNDER 37 C.F.R. 1.10

I hereby certify that the attached document is being deposited with the United States Postal Service, postage prepaid, on May 6, 1997 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service, mailing label number EM354713201US, in an envelope addressed to the Assistant Commissioner for Patents, Washington, DC 20231.


Michael J. Twomey

New Application Transmittal

Page 2

2. Benefit of Prior U.S. Application(s) (35 USC 120)

NOTE: If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., then check either the first option below or the second option below. If the second option is checked, the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION (S) IS CLAIMED must be completed and attached.

- ☐ The new application transmitted claims the benefit of prior U.S. application(s) and the priority information is contained in the enclosed new application
- ☐ The new application being transmitted claims the benefit of prior U.S. application(s) and enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

3. Papers Enclosed Which Are Required For Filing Date Under 37 CFR 1.53(b) (Regular) or 37 CFR 1.153 (Design) Application

33 Pages of specification
7 Pages of claims
1 Pages of Abstract
6 Sheets of drawing

- ☐ formal
- ☒ informal

WARNING: DO NOT submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. **Only one copy is required or desired.** Comments on proposed new 37 CFR 1.84. Notice of March 9, 1988 (1990 O.G. 57-62).

NOTE: "Identifying indicia such as the serial number, group and unit, title of the invention, attorney's docket number, inventor's name, number of sheets, etc., not to exceed 2-3/4 inches (7.0 cm.) in width may be placed in a centered location between the side edges within three fourths inch (19.1 mm.) of the top edge. Either this marking technique on the front of the drawing or the placement, although not preferred, of this information and the title of the invention on the back of the drawings is acceptable." Proposed 37 CFR 1.84(1). Notice of March 9, 1988 (1090 O.G. 67-62).

4. Additional papers enclosed

- ☐ Preliminary Amendment
- ☐ Information Disclosure Statement
- ☐ Form PTO-1449
- ☐ Citations
- ☐ Declaration of Biological Deposit

New Application Transmittal

Page 3

- ☐ Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.
- ☐ Authorization of Attorney(s) to Accept and Follow Instructions from Representative
- ☐ Special Comments
- ☐ Other

5. Declaration or oath

- ☒ Enclosed but unexecuted.
- ☐ Enclosed
executed by (check **all** applicable boxes)
 - ☐ inventor(s).
 - ☐ legal representative of inventor(s). 37 CFR 1.42 or 1.43
 - ☐ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.
 - ☐ this is the petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 is also attached.
See item 13 below for fee.
- ☐ Not Enclosed.

WARNING:

Where the filing is a completion in the U.S. of an International Application but where a declaration is not available or where the completion of the U.S. application contains subject matter in addition to the International Application the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.

- ☐ Application is made by a person authorized under 37 CFR 1.41(c) on behalf of all the above named inventor(s). The declaration or oath, along with the surcharge required by 37 CFR 1.16(e) can be filed subsequently.

NOTE: It is important that **all** the correct inventor(s) are named for filing under 37 CFR 1.41(c) and 1.53(b).

- ☐ Showing that the filing is authorized.
(Not required unless called into question. 37 CFR 1.41(d).)

6. Inventorship Statement

WARNING: If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.

The inventorship for all the claims in this application are:

- ☒ The same
- ☐ Are not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,
- ☐ is submitted.
- ☐ will be submitted.

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. A verified English translation of the non-English language application and the processing fee of \$130.00 required by 37 CFR 1.17(k) is required to be filed with the application or within such time as may be set by the Office. 37 CFR 1.52(d).

NOTE: A non-English oath or declaration in the form provided or approved by the PTO need not be translated. 37 CFR 1.69(b).

- ☒ English
- ☐ non-English
- ☐ the attached translation is a verified translation. 37 CFR 1.52(d).

8. Assignment

- ☐ An assignment of the invention to
- ☐ is (are) attached. A separate "ASSIGNMENT COVER LETTER ACCOMPANYING NEW PATENT APPLICATION" is also attached.
- ☒ will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters — one for the application and one for the assignment." Notice of May 4, 1990 (1114 D.G. 77-78).

9. Certified Copy

Certified copy(ies) of the application(s)

[illegible]

New Application Transmittal

Page 5

from which priority is claimed

☐ is (are) attached.

☐ will follow.

NOTE: The foreign application forming the basis for the claim for priority **must** be referred to in the **oath** or **declaration**. 37 CFR 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself entitled to priority from a prior foreign application then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. Fee Calculation (37 CFR 1.16)

A. ☒ **Regular application**

CLAIMS AS FILED

	Number Filed	Number Extra		Rate	Basic Fee 37 CFR 1.16(a) \$770.00
Total Claims (37 CFR 1.16 (c))	51	- 20 = 31	X	\$ 22.00	\$
Independent Claims (37 CFR 1.16 (b))	2	- 3 =	X	\$ 80.00	\$
Multiple Dependent Claim(s), If any (37 CFR 1.16(d))			+	\$ 260.00	\$

☐ Amendment canceling extra claims enclosed.

☐ Amendment deleting multiple-dependencies enclosed.

☒ Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims canceled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 CFR 1.16(d).

Filing Fee Calculation \$

B. ☐ **Design application**
(\$320.00--37 CFR 1.16(f))

Filing Fee Calculation \$

C. ☐ **Plant application**
(\$530.00--37 CFR 1.16(g))

Filing Fee Calculation \$

New Application Transmittal

Page 6

11. Small Entity Statement(s)

- ☐ Verified Statement(s) that this is a filing by a small entity under 37 CFR 1.9 and 1.27 is (are) attached.

Filing Fee Calculation (50% of A, B or C above) \$

NOTE: Any excess of the full fee paid will be refunded if a verified statement and a refund request are filed within 2 months of the date of timely payment of a full fee. 37 CFR 1.28(a).

12. Request for International-Type Search (37 CFR 1.104(d)) (complete, if applicable)

- ☐ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made At This Time

- ☒ Not Enclosed
- ☒ No filing fee is to be paid at this time. (This and the surcharge required by 37 CFR 1.16(e) can be paid subsequently.)
- ☐ Enclosed
- | | |
|---|----|
| <input type="checkbox"/> basic filing fee | \$ |
| <input type="checkbox"/> recording assignment
(\$40.00; 37 CFR 1.21(h)) | \$ |
| <input type="checkbox"/> petition fee for filing by other than all the
inventors or person on behalf of the
inventor where inventor refused to sign
or cannot be reached.
(\$130.00; 37 CFR 1.47 and 1.17(h)) | \$ |
| <input type="checkbox"/> for processing an application with a
specification in a non-English language.
(\$130.00; 37 CFR 1.52(d) and 1.17(k)) | \$ |
| <input type="checkbox"/> processing and retention fee (\$130.00;
37 CFR 1.53(d) and 1.21 (1)). | \$ |
| <input type="checkbox"/> fee for international-type search report
(\$40.00; 37 CFR 1.21 (e)). | \$ |

NOTE: 37 CFR 1.21(l) establishes a fee for processing and retaining any application which is abandoned for failing to complete the application pursuant to 37 CFR 1.53(d) and this, as well as the changes to 37 CFR 1.53 and 1.78, indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing

New Application Transmittal

Page 7

fee must be paid or the processing and retention fee of § 1.21(l) must be paid within 1 year from notification under § 53(d).

Total fees enclosed \$ **0.00**

14. Method of Payment of Fees

- ☐ Check in the amount of \$
- ☐ Charge Account No. 20-0531 in the amount of \$ _____. A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 CFR 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: If no fees are to be paid on filing the following items should not be completed.

WARNING: Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.

- ☐ The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No. 20-0531.

- ☐ 37 CFR 1.16(a),(f) or (g) (filing fees)
- ☐ 37 CFR 1.16(b),(c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims canceled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.16(d), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

- ☐ 37 CFR 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)
- ☐ 37 CFR 1.17 (application processing fees)

WARNING: While 37 CFR 1.17(a),(b), (c) and (d) deal with extensions of time under § 1.136(a) this authorization should be made only with the knowledge that: "Submission of the appropriate extension fee under 37 C.F.R. 1.136(a) is to no avail unless a request or petition for extension is filed." (Emphasis added). Notice of November 5, 1985 (1060 O.G. 27).

- ☐ 37 CFR 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 CFR 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance, 37 CFR 1.31(b).

NOTE: 37 CFR 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying, . . . issue fee". From the

New Application Transmittal

Page 8

wording of 37 CFR 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions As To Overpayment

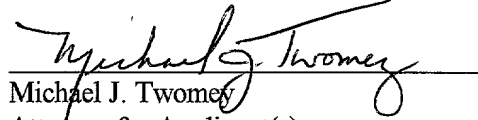
☐ credit Account No. 20-0531

☐ refund

Date: May 6, 1997

Reg. No. 38,349

Tel. No. (617) 248-7362


Michael J. Twomey
Attorney for Applicant(s)
Testa, Hurwitz, & Thibault, LLP
High Street Tower
125 High Street
Boston, Massachusetts 02110

☐ **Incorporation by reference of added pages**

Check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the **ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED**

☐ Plus Added Pages For New Application Transmittal Where Benefit of prior U.S. Application(s) Claimed

Number of pages added

☐ Plus Added Pages For Papers Referred To In Item 4 Above

Number of pages added

☐ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added

☐ **Statement Where No Further Pages Added**

(if no further pages form a part of this Transmittal then end this Transmittal with this page and check the following item)

☒ This transmittal ends with this page.

395MJT2054/18/356853-1

NOVEL THERAPIES FOR CHRONIC RENAL FAILURE

Field of the Invention

The present invention relates generally to methods of treatment for renal disease. In particular, the invention relates to methods of treatment for conditions which place mammals, including humans, in, or at risk of, chronic renal failure.

- 5 The methods involve the administration of certain proteins of, or based upon, the osteogenic protein/bone morphogenetic protein (OP/BMP) family within the TGF- β superfamily of proteins.

Background of the Invention

- 10 The mammalian renal system serves primary roles both in the removal of catabolic waste products from the bloodstream and in the maintenance of fluid and electrolyte balances in the body. Renal failures are, therefore, life-threatening conditions in which the build-up of catabolites and other toxins, and/or the development of significant imbalances in electrolytes or fluids, may lead to the failure of other major organs systems and death. As a general matter, renal failure
- 15 is classified as "acute" or "chronic." As detailed below, chronic renal failure is a debilitating and life-threatening disease for which no adequate treatment exists.

Chronic Renal Failure

- Chronic renal failure may be defined as a progressive, permanent and significant reduction of glomerular filtration rate (GFR) due to a significant and
- 20 continuing loss of nephrons. Chronic renal failure typically begins from a point at which a chronic renal insufficiency (i.e., a permanent decrease in renal function of at least 50-60%) has resulted from some insult to the renal tissues which has

caused a significant loss of nephron units. The initial insult may or may not have been associated with an episode of acute renal failure. Irrespective of the nature of the initial insult, chronic renal failure manifests a "final common path" of signs and symptoms as nephrons are progressively lost and GFR progressively declines. This
5 progressive deterioration in renal function is slow, typically spanning many years or decades in human patients, but seemingly inevitable.

The early stage of chronic renal failure typically begins when GFR has been reduced to approximately one-third of normal (e.g., 30-40 ml/min for an average human adult). As a result of the significant nephron loss, and in an apparent
10 "attempt" to maintain the overall GFR with fewer nephrons, the average single nephron GFR (SNGFR) is increased by adaptations of the remaining nephrons at both the structural and functional level. One structural manifestation of this adaptation, readily detectable by microscopic examination of biopsy samples, is a "compensatory hypertrophy" of both the glomeruli and the tubules of the kidney, a
15 process which literally increases the volume of filtrate which can be produced by each remaining nephron by literal enlargement of the glomeruli and tubules. Indeed, as a result of the hypertrophy or dilation of the collecting ducts, the urine of subjects with chronic renal failure often contains broad "casts," typically 2-6 times normal diameter, which aid in diagnosis and have also been referred to as
20 "renal failure casts." At the same time, there are functional changes in the remaining nephrons, such as decreased absorption or increased secretion of normally excreted solutes, which may be responses to hormonal or paracrine changes elsewhere in the body (e.g., increasing levels of parathyroid hormone (PTH) in response to changes in serum levels of calcium and phosphate).

25 These adaptations in early stage chronic renal failure are not successful in completely restoring GFR or other parameters of renal function and, in fact, subject the remaining nephrons to increased risk of loss. For example, the

increased SNGFR is associated with mechanical stresses on the glomerulus due to hypertension and hyperperfusion. The loss of integrity of podocyte junctures leads to increased permeability of the glomerulus to macromolecules or "leakiness" of the glomerular capsule. Proliferative effects are also observed in mesangial, epithelial and endothelial cells, as well as increases in the deposition of collagen and other matrix proteins. Sclerosis of both the glomeruli and tubules is another common symptom of the hypertrophied nephrons and the risk of coagulation in the glomerulus is increased. In particular, these adaptations of the remaining nephrons, by pushing the SNGFR well beyond its normal level, actually decrease the capacity of the remaining nephrons to respond to acute changes in water, solute, or acid loads and, therefore, actually increase the probability of additional nephron loss.

As chronic renal failure progresses, and GFR continues to decline to less than 10% of normal (e.g., 5-10 ml/min), the subject enters end-stage renal disease (ESRD). During this phase, the inability of the remaining nephrons to adequately remove waste products from the blood, while retaining useful products and maintaining fluid and electrolyte balance, leads to a rapid decline in which many organ systems, and particularly the cardiovascular system, may begin to fail. For example, BUN and creatinine levels may be expected to rise and, at BUN levels of 60-100 mg/dL and serum creatinine levels of 8-12 mg/dL, a uremic syndrome will typically develop in which the kidneys can no longer remove the end products of nitrogen metabolism. At this point, renal failure will rapidly progress to death unless the subject receives renal replacement therapy (i.e., chronic hemodialysis, continuous peritoneal dialysis, or kidney transplantation).

Approximately 600 patients per million receive chronic dialysis each year in the United States, at an average cost approaching \$60,000-\$80,000 per patient per year. Of the new cases of end-stage renal disease each year, approximately 28-

33% are due to diabetic nephropathy (or diabetic glomerulopathy or diabetic renal hypertrophy), 24-29% are due to hypertensive nephrosclerosis (or hypertensive glomerulosclerosis), and 15-22% are due to glomerulonephritis. The 5-year survival rate for all chronic dialysis patients is approximately 40%, but for patients
5 over 65, the rate drops to approximately 20%.

A need remains, therefore, for treatments which will prevent the progressive loss of renal function which has caused almost two hundred thousand patients in the United States alone to become dependent upon chronic dialysis, and which results in the premature deaths of tens of thousands each year.

10

Summary of the Invention

The present invention is directed to methods of treatment, and pharmaceutical preparations for use in the treatment, of mammalian subjects in, or at risk of, chronic renal failure, or at risk of the need for renal replacement therapy. Such subjects include subjects already afflicted with chronic renal failure, or which
15 have already received renal replacement therapy, as well as any subject reasonably expected to suffer a progressive loss of renal function associated with progressive loss of functioning nephron units. Whether a particular subject is at risk is a determination which may routinely be made by one of ordinary skill in the relevant medical or veterinary art. Subjects in, or at risk of, chronic renal failure, or at risk
20 of the need for renal replacement therapy, include but are not limited to the following: subjects which may be regarded as afflicted with chronic renal failure, end-stage renal disease, chronic diabetic nephropathy, hypertensive nephrosclerosis, chronic glomerulonephritis, hereditary nephritis, and/or renal dysplasia; subjects having a biopsy indicating glomerular hypertrophy, tubular
25 hypertrophy, chronic glomerulosclerosis, and/or chronic tubulointerstitial sclerosis; subjects having an ultrasound, MRI, CAT scan, or other non-invasive examination indicating renal fibrosis; subjects having an unusual number of broad casts present

in urinary sediment; subjects having a GFR which is chronically less than about 50%, and more particularly less than about 40%, 30% or 20%, of the expected GFR for the subject; human male subjects weighing at least about 50 kg and having a GFR which is chronically less than about 50 ml/min, and more particularly less than about 40 ml/min, 30 ml/min or 20 ml/min; human female subjects weighing at least about 40 kg and having a GFR which is chronically less than about 40 ml/min, and more particularly less than about 30 ml/min, 20 ml/min or 10 ml/min; subjects possessing a number of functional nephron units which is less than about 50%, and more particularly less than about 40%, 30% or 20%, of the number of functional nephron units possessed by a healthy but otherwise similar subject; subjects which have a single kidney; and subjects which are kidney transplant recipients.

The methods and compositions of this invention capitalize in part upon the discovery that certain proteins of eukaryotic origin, defined herein as renal therapeutic agents, and including members of the osteogenic protein/bone morphogenetic protein (OP/BMP) family of proteins, may be used in the treatment of subjects at risk, as defined herein, of chronic renal failure or the need for renal replacement therapy. Useful renal therapeutic agents include polypeptides, or functional variants of polypeptides, comprising at least the C-terminal six- or seven-cysteine domain of a mammalian protein selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP9, and proteins which exhibit at least 70% or, more preferably, 75% or 80% amino acid sequence homology with the amino acid sequence of the seven-cysteine domain of human OP-1; and which are (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the progressive loss of

renal function in a standard animal model of chronic renal failure, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, chronic renal failure.

- 5 The renal therapeutic agents of the invention may be administered by any route of administration which is compatible with the selected agent, and may be formulated with any pharmaceutically acceptable carrier appropriate to the route of administration. Preferred routes of administration are parenteral and, in particular, intravenous, intraperitoneal, and renal intracapsular. Treatments are also preferably conducted over an extended period on an outpatient basis. Daily
- 10 dosages of the renal therapeutic agents are expected to be in the range of about 0.01-1000 $\mu\text{g/kg}$ body weight, and more preferably about 10-300 $\mu\text{g/kg}$ body weight, although precise dosages will vary depending upon the particular renal therapeutic agent employed and the particular subject's medical condition and history.
- 15 The treatments of the present invention are useful in preventing, inhibiting or delaying the progressive loss of functional nephron units, and the consequent progressive loss of renal function, which typify chronic renal failure. As such they are of great value in preventing or delaying the need for chronic dialysis or renal replacement therapy in subjects with chronic renal insufficiency, or reducing the
- 20 necessary frequency of chronic renal dialysis in subjects with end-stage renal disease. As such, they are useful in prolonging the lives, and in maintaining the quality of life, of subjects at risk of, or already afflicted with, chronic renal failure.

Brief Description of the Figures

- Figure 1. This figure is a bar graph showing average serum creatinine
- 25 levels for groups of sham-operated ("SHAM") or partially nephrectomized ("Nx Contr" and "OP-1") rats. 5-6 months post-surgery, rats received injections of

vehicle only ("Nx control" and "SHAM") or 1, 3, 10 or 50 µg/kg body weight of soluble OP-1 ("OP-1") three times a week for 4-8 weeks.

Figure 2. This figure is a bar graph showing average serum urea levels for groups of sham-operated ("SHAM") or partially nephrectomized ("Nx Contr" and "OP-1") rats. 5-6 months post-surgery, rats received injections of vehicle only ("Nx control" and "SHAM") or 1, 3, 10 or 50 µg/kg body weight of soluble OP-1 ("OP-1") three times a week for 4-8 weeks.

Figure 3. Panels A-C of this figure are micrographs of renal tissue from rats at 10x magnification. (A) Tissue from sham-operated rat. (B) Tissue from rat in chronic renal failure after 5/6 nephrectomy (Nx control). (C) Tissue from rat treated with OP-1 after 5/6 nephrectomy.

Figure 4. Panels A-C of this figure are micrographs of renal tissue from rats at 40x magnification. (A) Tissue from sham-operated rat. (B) Tissue from rat in chronic renal failure after 5/6 nephrectomy (Nx control). (C) Tissue from rat treated with OP-1 after 5/6 nephrectomy.

Figure 5. This figure is a line graph showing average serum creatinine levels over 9 weeks for groups of partially nephrectomized rats. 2-3 weeks post-surgery, rats received injections of vehicle only ("Control") or 10 µg/kg body weight of soluble OP-1 ("OP-1") 3 times per week.

Figure 6. This figure is a line graph showing average creatinine clearance rates as a measure of GFR over 8 weeks for groups of partially nephrectomized rats. 2-3 weeks post-surgery, rats received injections of vehicle only ("Control") or 10 µg/kg body weight of soluble OP-1 ("OP-1") 3 times per week.

Detailed Description of the Invention

I. Definitions

In order to more clearly and concisely point out the subject matter of the claimed invention, the following definitions are provided for specific terms used in the following written description and appended claims.

Renal therapeutic agent. As used herein, the term "renal therapeutic agent" means a polypeptide, or a functional variant of a polypeptide, comprising at least the C-terminal six- or seven-cysteine domain of a mammalian protein selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP9, and proteins which exhibit at least 70% or, more preferably, 75% or 80% amino acid sequence homology with the amino acid sequence of the seven-cysteine domain of human OP-1; and which is (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the progressive loss of renal function in a standard animal model of chronic renal failure, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, chronic renal failure. As used herein, a percentage "homology" between two amino acid sequences indicates the percentage of amino acid residues which are identical or similar between the sequences and, as used herein, "similar" residues are "conservative substitutions" which fulfill the criteria defined for an "accepted point mutation" in Dayhoff et al. (1978), Atlas of Protein Sequence and Structure Vol. 5 (Suppl. 3), pp. 354-352, Natl. Biomed. Res. Found., Washington, D.C.

Therapeutic efficacy. As used herein, a renal therapeutic agent of the invention is said to have "therapeutic efficacy," and an amount of the agent is said to be "therapeutically effective," if administration of that amount of the agent is sufficient to cause a clinically significant improvement in a standard marker of renal function when administered to a mammalian subject (e.g., a human patient)

in, or at risk of, chronic renal failure. Such markers of renal function are well known in the medical literature and include, without being limited to, rates of increase in BUN levels, rates of increase in serum creatinine, static measurements of BUN, static measurements of serum creatinine, glomerular filtration rates (GFR), ratios of BUN/creatinine, serum concentrations of sodium (Na⁺), urine/plasma ratios for creatinine, urine/plasma ratios for urea, urine osmolality, daily urine output, and the like (see, for example, Brenner and Lazarus (1994), in Harrison's Principles of Internal Medicine, 13th edition, Isselbacher et al., eds., McGraw Hill Text, New York; Luke and Strom (1994), in Internal Medicine, 4th Edition, J.H. Stein, ed., Mosby-Year Book, Inc. St. Louis.)

Glomerular Filtration Rate (GFR). The "glomerular filtration rate" or "GFR" is proportional to the rate of clearance into urine of a plasma-borne substance which is not bound by serum proteins, is freely filtered across glomeruli, and is neither secreted nor reabsorbed by the renal tubules. Thus, as used herein, GFR preferably is defined by the following equation:

$$GFR = \frac{U_{conc} \times V}{P_{conc}}$$

where U_{conc} is the urine concentration of the marker, P_{conc} is the plasma concentration of the marker, and V is the urine flow rate in ml/min. Optionally, GFR is corrected for body surface area. Thus, the GFR values used herein may be regarded as being in units of ml/min/1.73m².

The preferred measure of GFR is the clearance of inulin but, because of the difficulty of measuring the concentrations of this substance, the clearance of creatinine is typically used in clinical settings. For example, for an average size, healthy human male (70 kg, 20-40 yrs), a typical GFR measured by creatinine clearance is expected to be approximately 125 ml/min with plasma concentrations of creatinine of 0.7-1.5 mg/dL. For a comparable, average size woman, a typical

GFR measured by creatinine clearance is expected to be approximately 115 ml/min with creatinine levels of 0.5-1.3 mg/dL. During times of good health, human GFR values are relatively stable until about age 40, when GFR typically begins to decrease with age. For subjects surviving to age 85 or 90, GFR may be reduced to
5 50% of the comparable values at age 40.

Expected Glomerular Filtration Rate (GFR_{exp}). An estimate of the "expected GFR" or " GFR_{exp} " may be provided based upon considerations of a subject's age, weight, sex, body surface area, and degree of musculature, and the plasma concentration of some marker compound (e.g., creatinine) as determined
10 by a blood test. Thus, as an example, an expected GFR or GFR_{exp} may be estimated as:

$$GFR_{exp} \approx \frac{(140 - age) \times weight (kg)}{72 \times P_{conc} (mg/dl)}$$

This estimate does not take into consideration such factors as surface area, degree of musculature, or percentage body fat. Nonetheless, using plasma creatinine
15 levels as the marker, this formula has been employed for human males as an inexpensive means of estimating GFR. Because creatinine is produced by striated muscle, the expected GFR or GFR_{exp} of human female subjects is estimated by the same equation multiplied by 0.85 to account for expected differences in muscle mass. (See Lemann, et al. (1990) Am. J. Kidney Dis. 16(3):236-243.)

20 Broad Cast. Microscopic examination of urinary sediment for the presence of formed elements is a standard procedure in urinalysis. Amongst the formed elements which may be present in urine are cylindrical masses of agglutinated materials that typically represent a mold or "cast" of the lumen of a distal convoluted tubule or collecting tubule. In healthy human subjects, such casts
25 typically have a diameter of 15-25 μm . In subjects with chronic renal failure, however, hypertrophy of the tubules may result in the presence of "broad casts" or

"renal failure casts" which are 2-6 times the diameter of normal casts and often have a homogeneous waxy appearance. Thus, as used herein, a "broad cast" means a urinary sediment cast having a diameter of 2-6 times normal, or about 30-150 μm for human casts.

- 5 Chronic. As used herein with respect to clinical indications such as urinary casts, measured GFR, or other markers of renal function, "chronic" means persisting for a period of at least three, and more preferably, at least six months. Thus, for example, a subject with a measured GFR chronically below 50% of GFR_{exp} is a subject in which the GFR has been measured and found to be below
- 10 50% of GFR_{exp} in at least two measurements separated by at least three, and more preferably, by at least six months, and for which there is no medically sound reason to believe that GFR was substantially (e.g., 10%) higher during the intervening period.

- Subjects in, or at risk of, chronic renal failure. As used herein, a subject is
- 15 said to be in, or at risk of, chronic renal failure, or at risk of the need for renal replacement therapy (i.e., chronic hemodialysis, continuous peritoneal dialysis, or kidney transplantation), if the subject is reasonably expected to suffer a progressive loss of renal function associated with progressive loss of functioning nephron units. Whether a particular subject is in, or at risk of, chronic renal failure is a
- 20 determination which may routinely be made by one of ordinary skill in the relevant medical or veterinary art. Subjects in, or at risk of, chronic renal failure, or at risk of the need for renal replacement therapy, include but are not limited to the following: subjects which may be regarded as afflicted with chronic renal failure, end-stage renal disease, chronic diabetic nephropathy, hypertensive
- 25 nephrosclerosis, chronic glomerulonephritis, hereditary nephritis, and/or renal dysplasia; subjects having a biopsy indicating glomerular hypertrophy, tubular hypertrophy, chronic glomerulosclerosis, and/or chronic tubulointerstitial sclerosis;

subjects having an ultrasound, MRI, CAT scan, or other non-invasive examination indicating renal fibrosis; subjects having an unusual number of broad casts present in urinary sediment; subjects having a GFR which is chronically less than about 50%, and more particularly less than about 40%, 30% or 20%, of the expected

5 GFR for the subject; human male subjects weighing at least about 50 kg and having a GFR which is chronically less than about 50 ml/min, and more particularly less than about 40 ml/min, 30 ml/min or 20 ml/min; human female subjects weighing at least about 40 kg and having a GFR which is chronically less than about 40 ml/min, and more particularly less than about 30 ml/min, 20 ml/min or 10

10 ml/min; subjects possessing a number of functional nephron units which is less than about 50%, and more particularly less than about 40%, 30% or 20%, of the number of functional nephron units possessed by a healthy but otherwise similar subject; subjects which have a single kidney; and subjects which are kidney transplant recipients.

15 II. Description of the Preferred Embodiments

A. General

The present invention depends, in part, upon the surprising discovery that administration of certain protein-based renal therapeutic agents to subjects in, or at risk of, chronic renal failure, can reduce mortality and/or morbidity rates, and

20 prevent, inhibit, delay or alleviate the progressive loss of renal function which characterizes chronic renal failure. Alternatively, or in addition, administration of the renal therapeutic agents of the present invention can prevent, inhibit or delay the progressive loss of functional nephron units and the progressive decline in glomerular filtration rate (GFR) which slowly but inevitably leads to the need for

25 renal replacement therapy (i.e., renal transplant or chronic dialysis) or death. In preferred embodiments, the therapeutic agents of the invention are members of the

osteogenic protein/bone morphogenetic protein (OP/BMP) family within the TGF- β superfamily of proteins.

B. Renal Therapeutic Agents

The renal therapeutic agents of the present invention are naturally
5 occurring proteins, or functional variants of naturally occurring proteins, in the
osteogenic protein/bone morphogenetic protein (OP/BMP) family within the TGF- β
superfamily of proteins. That is, these proteins form a distinct subgroup,
referred to herein as the "OP/BMP family," within the loose evolutionary grouping
of sequence-related proteins known as the TGF- β superfamily. Members of this
10 protein family comprise secreted polypeptides that share common structural
features, and that are similarly processed from a pro-protein to yield a carboxy-
terminal mature protein. Within the mature protein, all members share a conserved
pattern of six or seven cysteine residues defining a 97-106 amino acid domain, and
the active form of these proteins is either a disulfide-bonded homodimer of a single
15 family member, or a heterodimer of two different members (see, e.g., Massague
(1990), Annu. Rev. Cell Biol. 6:597; Sampath et al. (1990), J. Biol. Chem.
265:13198). For example, in its mature, native form, natural-sourced human OP-1
is a glycosylated dimer typically having an apparent molecular weight of about
30-36 kDa as determined by SDS-PAGE. When reduced, the 30 kDa protein
20 gives rise to two glycosylated peptide subunits having apparent molecular weights
of about 16 kDa and 18 kDa. The unglycosylated protein has an apparent
molecular weight of about 27 kDa. When reduced, the 27 kDa protein gives rise
to two unglycosylated polypeptide chains, having molecular weights of about 14
kDa to 16 kDa.
25 Typically, the naturally occurring OP/BMP proteins are translated as a
precursor, having an N-terminal signal peptide sequence, a "pro" domain, and a
"mature" protein domain. The signal peptide is typically less than 30 residues, and

is cleaved rapidly upon translation at a cleavage site that can be predicted using the method of Von Heijne (1986), Nucleic Acids Research 14:4683-4691. The "pro" domain is variable both in sequence and in length, ranging from approximately 200 to over 400 residues. The pro domain is cleaved to yield the "mature" C-terminal domain of approximately 115-180 residues, which includes the conserved six- or seven-cysteine C-terminal domain of 97-106 residues. As used herein, the "pro form" of an OP/BMP family member refers to a protein comprising a folded pair of polypeptides, each comprising a pro domain in either covalent or noncovalent association with the mature domains of the OP/BMP polypeptide. Typically, the pro form of the protein is more soluble than the mature form under physiological conditions. The pro form appears to be the primary form secreted from cultured mammalian cells. The "mature form" of the protein refers to mature C-terminal domain which is not associated, either covalently or noncovalently, with the pro domain. Any preparation of OP-1 is considered to contain mature form when the amount of pro domain in the preparation is no more than 5% of the amount of "mature" C-terminal domain.

OP/BMP family members useful herein include any of the known naturally-occurring native proteins including allelic, phylogenetic counterpart and other variants thereof, whether naturally-sourced or biosynthetically produced (e.g., including "muteins" or "mutant proteins"), as well as new, active members of the OP/BMP family of proteins.

Particularly useful sequences include those comprising the C-terminal seven cysteine domains of mammalian, preferably human, human OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP8 and BMP9. Other proteins useful in the practice of the invention include active forms of GDF-5, GDF-6, GDF-7, DPP, Vg1, Vgr-1, 60A, GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, BMP10, BMP11, BMP13, BMP15, UNIVIN, NODAL, SCREW, ADMP or NURAL and amino

acid sequence variants thereof. In one currently preferred embodiment, the renal therapeutic agents of the invention are selected from any one of: OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, and BMP9.

- Publications disclosing these sequences, as well as their chemical and
- 5 physical properties, include: OP-1 and OP-2: U.S. Pat. No. 5,011,691, U.S. Pat. No. 5,266,683, and Ozkaynak et al. (1990), EMBO J. 9:2085-2093; OP-3: WO94/10203; BMP2, BMP3, and BMP4: U.S. Pat. No. 5,013,649, WO91/18098, WO88/00205, and Wozney et al. (1988), Science 242:1528-1534; BMP5 and BMP6: WO90/11366 and Celeste et al. (1991), Proc. Natl. Acad. Sci. (USA)
- 10 87:9843-9847; Vgr-1: Lyons et al. (1989), Proc. Natl. Acad. Sci. (USA) 86: 4554-4558; DPP: Padgett et al. (1987), Nature 325:81-84; Vgl: Weeks (1987), Cell 51:861-867; BMP-9: WO95/33830; BMP10: WO94/26893; BMP-11: WO94/26892; BMP12: WO95/16035; BMP-13: WO95/16035; GDF-1: WO92/00382 and Lee et al. (1991), Proc. Natl. Acad. Sci. (USA) 88:4250-4254;
- 15 GDF-8: WO94/21681; GDF-9: WO94/15966; GDF-10: WO95/10539; GDF-11: WO96/01845; BMP-15: WO96/36710; MP121: WO96/01316; GDF-5 (CDMP-1, MP52): WO94/15949, WO96/14335, WO93/16099 and Storm et al. (1994), Nature 368:639-643; GDF-6 (CDMP-2, BMP13): WO95/01801, WO96/14335 and WO95/10635; GDF-7 (CDMP-3, BMP12): WO95/10802 and WO95/10635;
- 20 BMP-3b: Takao, et al. (1996), Biochem. Biophys. Res. Comm. 219:656-662; GDF-3: WO94/15965; 60A: Blaster et al. (1993), Cell 73:687-702 and GenBank accession number L12032. In another embodiment, useful proteins include biologically active biosynthetic constructs, including novel biosynthetic proteins and chimeric proteins designed using sequences from two or more known
- 25 OP/BMP family proteins. See also the biosynthetic constructs disclosed in U.S. Pat. No. 5,011,691, the disclosure of which is incorporated herein by reference (e.g., COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16).

In other preferred embodiments, the renal therapeutic agents useful herein include therapeutically effective proteins in which the amino acid sequences comprise a sequence sharing at least 70% amino acid sequence "homology" and, preferably, 75% or 80% homology with the C-terminal seven cysteine domain

5 present in the active forms of human OP-1 (i.e., residues 330-431, as shown in SEQ ID NO: 2 of U.S. Pat. No. 5,266,683). In other preferred embodiments, the renal therapeutic agents useful herein include therapeutically effective proteins in which the amino acid sequences comprise a sequence sharing at least 60% amino acid sequence identity and, preferably, 65% or 70% identity with the C-terminal

10 seven cysteine domain present in the active forms of human OP-1. Thus, a candidate amino acid sequence thought to have therapeutic efficacy in the present invention can be aligned with the amino acid sequence of the C-terminal seven cysteine domain of human OP-1 using the method of Needleman et al. (1970), J. Mol. Biol. 48:443-453, implemented conveniently by computer programs such as

15 the Align program (DNASTar, Inc.). As will be understood by those skilled in the art, homologous or functionally equivalent sequences include functionally equivalent arrangements of the cysteine residues within the conserved cysteine skeleton, including amino acid insertions or deletions which alter the linear arrangement of these cysteines, but do not materially impair their relationship in

20 the folded structure of the dimeric protein, including their ability to form such intra- or inter-chain disulfide bonds as may be necessary for biological activity. Therefore, internal gaps and amino acid insertions in the candidate sequence are ignored for purposes of calculating the level of amino acid sequence homology or identity between the candidate and reference sequences.

25 "Amino acid sequence homology" is understood herein to include both amino acid sequence identity and similarity. Thus, as used herein, a percentage "homology" between two amino acid sequences indicates the percentage of amino

acid residues which are identical or similar between the sequences. "Similar" residues are "conservative substitutions" which fulfill the criteria defined for an "accepted point mutation" in Dayhoff et al. (1978), Atlas of Protein Sequence and Structure Vol. 5 (Suppl. 3), pp. 354-352, Natl. Biomed. Res. Found., Washington,

- 5 D.C. Thus, "conservative substitutions" are residues that are physically or functionally similar to the corresponding reference residues, having similar size, shape, electric charge, and/or chemical properties such as the ability to form covalent or hydrogen bonds, or the like. Examples of conservative substitutions include the substitution of one amino acid for another with similar characteristics,
- 10 e.g., substitutions within the following groups: (a) valine, glycine; (b) glycine, alanine; (c) valine, isoleucine, leucine; (d) aspartic acid, glutamic acid; (e) asparagine, glutamine; (f) serine, threonine; (g) lysine, arginine, methionine; and (h) phenylalanine, tyrosine. The term "conservative substitution" or "conservative variation" also includes the use of a substituted amino acid in place of an
- 15 unsubstituted parent amino acid in a given polypeptide chain, provided that the resulting substituted polypeptide chain also has therapeutic efficacy in the present invention.

The renal therapeutic agents of the invention are also characterized by biological activities which may be readily ascertained by those of ordinary skill in

20 the art. Specifically, a renal therapeutic agent of the present invention is (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the progressive loss of renal function in a standard animal

25 model of chronic renal failure, or (c) capable of causing a clinically significant

improvement in a standard marker of renal function when administered to a mammal in, or at risk of, chronic renal failure.

The Reddi-Sampath ectopic bone assay is well known in the art as an assay of chondrogenic activity. The assay, which can be easily performed, is described and discussed in, for example, Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603; and Wozney (1989), "Bone Morphogenetic Proteins," Progress in Growth Factor Research 1:267-280. Many equivalent assays, using other animals and tissue sites, may be employed or developed by those of skill in the art to evaluate the biological activity of the renal therapeutic agents of the present invention. See, for example, the bioassays described in U.S. Pat. No. 5,226,683.

The renal therapeutic agents of the present invention also may be tested in animal models of chronic renal failure. Mammalian models of chronic renal failure in, for example, mice, rats, guinea pigs, cats, dogs, sheep, goats, pigs, cows, horses, and non-human primates, may be created by causing an appropriate direct or indirect injury or insult to the renal tissues of the animal. Animal models of chronic renal failure may, for example, be created by performing a partial (e.g., 5/6) nephrectomy which reduces the number of functioning nephron units to a level which initiates compensatory renal hypertrophy, further nephron loss, and the progressive decline in renal function which characterizes chronic renal failure.

Finally, the renal therapeutic agents of the present invention may be evaluated for their therapeutic efficacy in causing a clinically significant improvement in a standard marker of renal function when administered to a mammalian subject (e.g., a human patient) in, or at risk of, chronic renal failure. Such markers of renal function are well known in the medical literature and include, without being limited to, rates of increase in BUN levels, rates of increase in serum creatinine, static measurements of BUN, static measurements of serum

creatinine, glomerular filtration rates (GFR), ratios of BUN/creatinine, serum concentrations of sodium (Na⁺), urine/plasma ratios for creatinine, urine/plasma ratios for urea, urine osmolality, daily urine output, and the like (see, for example, Brenner and Lazarus (1994), in Harrison's Principles of Internal Medicine, 13th edition, Isselbacher et al., eds., McGraw Hill Text, New York; Luke and Strom (1994), in Internal Medicine, 4th Edition, J.H. Stein, ed., Mosby-Year Book, Inc. St. Louis.)

The renal therapeutic agents contemplated herein can be expressed from intact or truncated genomic or cDNA or from synthetic DNAs in prokaryotic or eukaryotic host cells. The dimeric proteins can be isolated from the culture media and/or refolded and dimerized in vitro to form biologically active compositions. Heterodimers can be formed in vitro by combining separate, distinct polypeptide chains. Alternatively, heterodimers can be formed in a single cell by co-expressing nucleic acids encoding separate, distinct polypeptide chains. See, for example, WO93/09229, or U.S. Pat. No. 5,411,941, for several exemplary recombinant heterodimer protein production protocols. Currently preferred host cells include, without limitation, prokaryotes including E. coli, or eukaryotes including yeast, Saccharomyces, insect cells, or mammalian cells, such as CHO, COS or BSC cells. One of ordinary skill in the art will appreciate that other host cells can be used to advantage. Detailed descriptions of the proteins useful in the practice of this invention, including how to make, use and test them for chondrogenic activity, are disclosed in numerous publications, including U.S. Pat. Nos. 5,266,683 and 5,011,691, the disclosures of which are herein incorporated by reference.

C. Subjects for Treatment

As a general matter, the methods of the present invention may be utilized for any mammalian subject in, or at risk of, chronic renal failure, or at risk of the need for renal replacement therapy (i.e., chronic dialysis or renal transplant).

Mammalian subjects which may be treated according to the methods of the invention include, but are not limited to, human subjects or patients. In addition, however, the invention may be employed in the treatment of domesticated mammals which are maintained as human companions (e.g., dogs, cats, horses), which have significant commercial value (e.g., dairy cows, beef cattle, sporting animals), which have significant scientific value (e.g., captive or free specimens of endangered species), or which otherwise have value. In addition, as a general matter, the subjects for treatment with the methods of the present invention need not present indications for treatment with the renal therapeutic agents of the invention other than those indications associated with risk of chronic renal failure. That is, the subjects for treatment are expected to be otherwise free of indications for treatment with the renal therapeutic agents of the invention. In some number of cases, however, the subjects may present with other symptoms (e.g., osteodystrophy) for which treatment with the agents of the present invention would be indicated. In such cases, the treatment should be adjusted accordingly so to avoid excessive dosing.

One of ordinary skill in the medical or veterinary arts is trained to recognize subjects which may be at a substantial risk of chronic renal failure, or at substantial risk of the need for renal replacement therapy. In particular, clinical and non-clinical trials, as well as accumulated experience, relating to the presently disclosed and other methods of treatment, are expected to inform the skilled practitioner in deciding whether a given subject is in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, and whether any particular treatment is best suited to the subject's needs, including treatment according to the present invention.

As a general matter, a mammalian subject may be regarded as being in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, if

that subject has already been diagnosed as afflicted with, or would be regarded as being afflicted with, a condition which typically leads to progressive loss of renal function associated with progressive loss of functioning nephron units. Such conditions include, but are not limited to, chronic renal failure, end-stage renal disease, chronic diabetic nephropathy, hypertensive nephrosclerosis, chronic glomerulonephritis, hereditary nephritis, renal dysplasia and the like. These, and other diseases and conditions known in the art, typically lead to a progressive loss of functioning nephrons and to the onset of chronic renal failure.

Frequently, one of skill in the medical or veterinary arts may base a prognosis, diagnosis or treatment decision upon an examination of a renal biopsy sample. Such biopsies provide a wealth of information useful in diagnosing disorders of the kidney but, due to the invasiveness of the procedure, and the additional trauma to a presumably unhealthy kidney, may not be appropriate for all subjects. Nonetheless, subjects in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, may be recognized by histological indications from renal biopsies including, but not limited to, glomerular hypertrophy, tubular hypertrophy, glomerulosclerosis, tubulointerstitial sclerosis, and the like.

Less invasive techniques for assessing kidney morphology include MRI, CAT and ultrasound scans. Scanning techniques are also available which employ contrasting or imaging agents (e.g., radioactive dyes) but, it should be noted, some of these are particularly toxic to renal tissues and structures and, therefore, their use may be ill-advised in subjects in, or at risk of, chronic renal failure. Such non-invasive scanning techniques may be employed to detect conditions such as renal fibrosis or sclerosis, focal renal necrosis, renal cysts, and renal gross hypertrophy which will place a subject in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy.

Quite frequently, prognosis, diagnosis and/or treatment decisions are based upon clinical indications of renal function. One such indication is the presence in urinary sediment of an unusual number of "broad" or "renal failure" casts, which is indicative of tubular hypertrophy and suggests the compensatory renal hypertrophy which typifies chronic renal failure. A better indication of renal function is the glomerular flow rate (GFR), which can be measured directly by quantifying the rate of clearance of particular markers, or which may be inferred from indirect measurements.

It should be noted that the present invention is not directed to the measurement of GFR or to the diagnosis of chronic renal failure. The methods of treatment of the present invention need not, therefore, be restricted to subjects presenting with any particular measures of GFR, or any other particular marker of renal function. Indeed, it is not necessary that the GFR of a subject, or any other particular marker of renal function, be determined before practicing the treatments of the present invention. Nonetheless, the measurement of GFR is considered to be a preferred means of assessing renal function.

As is well known in the art, GFR reflects the rate of clearance of a reference or marker compound from the plasma to the urine. The marker compound to be considered is typically one which is freely filtered by the glomeruli, but which is not actively secreted or reabsorbed by the renal tubules, and which is not significantly bound by circulating proteins. The rate of clearance is typically defined by the formula, presented above, which relates the volume of urine produced in a twenty-four period, and the relative concentrations of the marker in the urine and plasma. To be more accurate, the GFR should also be corrected for body surface area. The "gold standard" reference compound is inulin because of its filtration properties and lack of serum-binding. The concentration of this compound is, however, difficult to quantify in blood or urine. The clearance

rates of other compounds, including p-aminohippurate (PAH) and creatinine, are therefore often used instead of inulin. In addition, various formulas are often employed which seek to simplify the estimation of actual GFR by omitting considerations of actual urine concentrations of the marker, actual daily volumes of urine produced, or actual body surface area. These values may be replaced by estimates based on other factors, by baseline values established for the same subject, or by standard values for similar subjects. These estimates should be used with caution, however, as they may entail inappropriate assumptions based upon the renal function of normal or healthy subjects.

10 Various methods and formulas have been developed in the art which describe an expected value of GFR for a healthy subject with certain characteristics. In particular, formulas are available which provide an expected value of the GFR based upon plasma creatinine levels, age, weight and sex. One such formula for an expected GFR is presented above. Other formulas may, of course, be employed and tables of standard values may be produced for subjects of a given age, weight, sex, and/or plasma creatinine concentration. Newer methods of measuring or estimating GFR (e.g., using NMR or MRI technologies) are also now available in the art and may be used in accordance with the present invention (see, e.g., U.S. Pat. Nos. 5,100,646 and 5,335,660).

20 As a general matter, irrespective of the manner in which GFR is measured or estimated, a subject may be considered to be in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, when the subject has a GFR which is chronically less than about 50% of the expected GFR for that subject. The risk is considered greater as the GFR falls lower. Thus, a subject is increasingly considered at risk if the subject has a GFR which is chronically less than about 40%, 30% or 20% of the expected GFR.

As a general matter, irrespective of the manner in which GFR is measured or estimated, a human male subject weighing at least about 50 kg may be considered to be in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, when the subject has a GFR which is chronically less than
5 about 50 ml/min. The risk is considered greater as the GFR falls lower. Thus, a subject is increasingly considered at risk if the subject has a GFR which is chronically less than about 40, 30 or 20 ml/min.

As a general matter, irrespective of the manner in which GFR is measured or estimated, a human female subject weighing at least about 40 kg may be
10 considered to be in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, when the subject has a GFR which is chronically less than about 40 ml/min. The risk is considered greater as the GFR falls lower. Thus, a subject is increasingly considered at risk if the subject has a GFR which is chronically less than about 30, 20 or 10 ml/min.

15 By employing a variety of methods, including the histological examinations, non-invasive scanning procedures, evaluations of clinical indicators, and other techniques described above and known in the art, those in the medical and veterinary arts may provide estimates of either the number of functioning nephron units which a subject possesses, or the percentage of functioning nephron
20 units which a subject possesses relative to a healthy but otherwise similar subject (e.g., a conspecific subject of approximately the same age, weight, and sex). Thus, for example, a biopsy may reveal a decrease in the density of functional nephrons, or imaging with filtered agents may indicate losses of functional renal tissue and/or filtering capacity. Such measures or estimates provide another means of
25 expressing when a subject is in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy. Thus, as a general matter, a subject may be regarded to be in, or at risk of, chronic renal failure, or at risk of needing renal

replacement therapy, if that subject possesses a number of functional nephron units which is less than about 50% of the number of functional nephron units of a healthy, but otherwise similar, subject. As above, the risk is considered greater as the number of functional nephrons decreases further. Thus, a subject is

5 increasingly considered at risk if the subject has a number of functional nephrons which is less than about 40, 30 or 20% of the number for a similar but healthy subject.

Finally, it should be noted that subjects possessing a single kidney, irrespective of the manner of loss of the other kidney (e.g., physical trauma,

10 surgical removal, birth defect), may be considered to be prima facie at risk of chronic renal failure, or the need for renal replacement therapy. This is particularly true for those subjects in which one kidney has been lost due to a disease or condition which may afflict the remaining kidney. Similarly, subjects which are already recipients of a renal transplant, or which are already receiving chronic

15 dialysis (e.g., chronic hemodialysis or continuous ambulatory peritoneal dialysis) may be considered prima facie to be at risk of chronic renal failure, or the need for further renal replacement therapy.

D. Formulations and Methods of Treatment

20 The renal therapeutic agents of the present invention may be administered by any route which is compatible with the particular renal therapeutic agent employed. Thus, as appropriate, administration may be oral or parenteral, including intravenous, intraperitoneal, and renal intracapsular routes of administration. In addition, administration may be by periodic injections of a bolus

25 of the renal therapeutic agent, or may be made more continuous by intravenous or intraperitoneal administration from a reservoir which is external (e.g., an i.v. bag) or internal (e.g., a bioerodable implant or implanted pump).

The renal therapeutic agents of the invention may be provided to an individual by any suitable means, preferably directly (e.g., locally, as by injection or topical administration to a tissue locus) or systemically (e.g., parenterally or orally). Where the agent is to be provided parenterally, such as by intravenous, 5 subcutaneous, or intramuscular, administration, the agent preferably comprises part of an aqueous solution. The solution is physiologically acceptable so that in addition to delivery of the desired agent to the subject, the solution does not otherwise adversely affect the subject's electrolyte and/or volume balance. The aqueous medium for the agent thus may comprise normal physiologic saline (e.g., 10 9.85% NaCl, 0.15M, pH 7-7.4).

If desired, a given renal therapeutic agent or other agent may be made more soluble by association with a suitable molecule. For example, association of a mature OP/BMP dimer with an OP/BMP pro domain results in the pro form of the renal therapeutic agent which typically is more soluble or dispersible in 15 physiological solutions than the corresponding mature form. In fact, endogenous members of the OP/BMP family are thought to be transported (e.g., secreted and circulated) in the mammalian body in this form. This soluble form of the protein can be obtained from culture medium of OP/BMP-secreting mammalian cells, e.g., cells transfected with nucleic acid encoding and competent to express the protein. 20 Alternatively, a soluble species can be formulated by complexing the mature dimer (or an active fragment thereof) with a pro domain or a solubility-enhancing fragment thereof (described more fully below). Another molecule capable of enhancing solubility and particularly useful for oral administrations, is casein. For example, addition of 0.2% casein increases solubility of the mature active form of 25 OP-1 by 80%. Other components found in milk and/or various serum proteins also may be useful.

Useful solutions for parenteral administration may be prepared by any of the methods well known in the pharmaceutical art, described, for example, in Remington's Pharmaceutical Sciences (Gennaro, A., ed.), Mack Pub., 1990.

Alternatively, the agents described herein may be administered orally. Oral administration of proteins as therapeutics generally is not practiced as most proteins are readily degraded by digestive enzymes and acids in the mammalian digestive system before they can be absorbed into the bloodstream. However, the renal therapeutic agents described herein typically are acid stable and protease-resistant (see, for example, U.S. Pat. No. 4,968,590). In addition, at least one of these renal therapeutic agents, OP-1, has been identified in mammary gland extract, colostrum and 57-day milk. Moreover, the OP-1 purified from mammary gland extract is therapeutically efficacious and also is detected in the bloodstream. Finally, soluble form OP-1, e.g., mature OP-1 associated with the pro domain, is therapeutically efficacious. These findings, as well as those disclosed in the examples below, indicate that oral and parenteral administration are viable means for administering the renal therapeutic agents of the invention to an individual. In addition, while the mature forms of certain renal therapeutic agents described herein typically are sparingly soluble, the form found in milk (and mammary gland extract and colostrum) is readily soluble, probably by association of the mature, therapeutically efficacious form with part or all of the pro domain of the intact sequence and/or by association with one or more milk components. Accordingly, the compounds provided herein also may be associated with molecules capable of enhancing their solubility in vitro or in vivo.

The compounds provided herein also may be associated with molecules capable of targeting the renal therapeutic agent to the desired tissue. For example, an antibody, antibody fragment, or other binding protein that interacts specifically with a surface molecule on cells of the desired tissue, may be used. Useful

targeting molecules may be designed, for example, using the single chain binding site technology disclosed, for example, in U.S. Pat. No. 5,091,513.

As will be appreciated by one of ordinary skill in the art, the formulated compositions contain therapeutically effective amounts of the renal therapeutic agent. That is, they contain amounts which provide appropriate concentrations of the agent to the renal tissues for a time sufficient to prevent, inhibit, delay or alleviate permanent or progressive loss of renal function, or otherwise provide therapeutic efficacy. As will be appreciated by those skilled in the art, the concentration of the compounds described in a therapeutic composition of the present invention will vary depending upon a number of factors, including the biological efficacy of the selected agent, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, the formulation of the compound excipients, the administration route, and the treatment envisioned, including whether the active ingredient will be administered directly into a kidney or renal capsule, or whether it will be administered systemically. The preferred dosage to be administered also is likely to depend on such variables such as the condition of the renal tissues, extent of renal function loss, and the overall health status of the particular subject. Dosages may be administered continuously, or daily, but it is currently preferred that dosages be administered once, twice or three times per week for as long as satisfactory response persists (as measured, for example, by stabilization and/or improvement of renal function by appropriate medical markers and/or quality of life indices) Less frequent dosages, for example monthly dosages, may also be employed. For subjects which would otherwise require continuous, bi-weekly or tri-weekly hemodialysis sessions, continuous, bi-weekly or tri-weekly intravenous or intraperitoneal infusions are not considered unduly inconvenient. In addition, in order to facilitate frequent infusions, implantation of

a semi-permanent stent (e.g., intravenous, intraperitoneal or intracapsular) may be advisable.

The renal therapeutic agents of the invention may, of course, be administered alone or in combination with other molecules known to be beneficial in the treatment of the conditions described herein. When used in combination with other agents, it may be advisable to alter the dosages of the renal therapeutic agents of the present invention accordingly.

Practice of the invention, including additional preferred aspects and embodiments thereof, will be still more fully understood from the following examples, which are presented herein for illustration only and should not be construed as limiting the invention in any way.

Examples

Rat Remnant Kidney Model

A rat partial (5/6) nephrectomy or rat remnant kidney model (RRKM) model was employed essentially as described (Vukicevic, et al. (1987) J. Bone Mineral Res. 2:533). Male rats (2-3 months old, weighing about 150-200 g) were subjected to unilateral nephrectomy (either left or right kidney). After approximately one week, 2/3 of the remaining kidney was surgically removed. Immediately following surgery, plasma creatinine and BUN levels rise dramatically due to the loss of renal mass and function. Over the next several weeks of this "acute" failure phase, plasma creatinine and BUN levels of surviving animals decline somewhat toward normal values but remain elevated. Renal function then appears to remain relatively constant or stable for a period of variable duration. After this point, the animals enter a period of chronic renal failure in which there is an essentially linear decline in renal function ending in death.

As surgical controls, additional rats were subjected to a "sham" operation in which the kidneys were decapsulated but no renal tissue was removed.

Intervention Model for Chronic Renal Failure

In this model, both nephrectomized and sham-operated rats were maintained for approximately 5-6 months after surgery. At this point, surviving nephrectomized animals were past the stable phase and had entered chronic renal failure.

Rats were divided into 8 groups with 12 rats in each group. Two groups of nephrectomized rats were used as controls (Nx controls), with one of those groups receiving no treatment at all, while the other received injections of only the vehicle buffer. In addition, two groups of sham-operated rats were used as controls (sham controls), with one group receiving only the vehicle buffer, while the other received soluble OP-1 (sOP-1) at 10 µg/kg body weight. Four experimental groups of nephrectomized rats were employed, receiving sOP-1 at 1, 3, 10 or 50 µg/kg body weight by intraperitoneal injection (OP-1 Nx animals). OP-1 treated and vehicle-only rats received three injections per week for 4-8 weeks. Total injection volume was 300 µl. No statistically significant differences were observed between the two Nx control groups or between the two sham control groups.

Compared to the sham group receiving only vehicle, the Nx control receiving only vehicle demonstrated significantly ($p < 0.01$) elevated serum creatinine (Figure 1) at the end of the study, indicating a significant loss of renal function. Although nephrectomized rats treated with either 1 or 3 µg/kg body weight sOP-1 did not show significantly reduced serum creatinine when compared to the Nx control, nephrectomized rats treated with sOP-1 at doses of 10 or 50 µg/kg body weight showed significant ($p < 0.05$) reductions in creatinine values (Figure 1). Similar results were observed for serum urea levels: Although nephrectomized rats treated with either 1 or 3 µg/kg body weight sOP-1 did not show significantly reduced serum urea when compared to the Nx control,

nephrectomized rats treated with sOP-1 at doses of 10 or 50 µg/kg body weight showed significant ($p < 0.01$) reductions in serum urea values (Figure 2). All nephrectomized rats showed significantly ($p < 0.01$) higher serum urea when compared to the sham-operated rats (Figure 2).

- 5 Histological observations indicate that, in contrast to the vehicle treated Nx control group, OP-1 treated nephrectomized rats exhibit relatively normal glomerular histology,. Figure 3, for example, shows typical renal samples from (A) normal rat kidney, (B) untreated Nx control animals, and (C) OP-1 treated nephrectomized rats under low magnification (10x). Figure 4 shows similar
10 samples under higher magnification (40x). Histomorphometric analysis indicates that OP-1 Nx rats showed reduced incidence of glomerular sclerosis and loop collapse, relatively scattered sclerosis and microaneurysms, and more viable glomeruli compared to Nx control rats (Table 1).

None of the rats died in any group during this study.

15 Prophylactic Model for Chronic Renal Failure

- Rats were subjected to partial nephrectomies or sham-operated as described above. In this model, in order to test the ability of OP/BMP renal therapeutic agents to prevent, inhibit or delay the initiation of chronic renal failure, the rats were allowed to recover for approximately two weeks after surgery before
20 initiation of OP-1 therapy. At this point, surviving animals were past the acute renal failure phase and had not yet entered chronic renal failure.

- Rats were divided into two groups of 15-20 rats. One group received only vehicle buffer (Nx control) whereas the other received OP-1 treatment at 10 µg/kg body weight given intraperitoneally three times per week. Administration of OP-1
25 or vehicle continued for a period of approximately 8-9 weeks.

During weeks 1-5 of treatment, both groups showed elevated serum creatinine ($> 100 \mu\text{mol/L}$) relative to sham-operated controls ($35 \pm 7 \mu\text{mol/L}$). At

about 5 weeks, both groups began to show a rise in serum creatinine suggesting the onset of progressive or chronic renal failure. The rise in serum creatinine was, however, markedly less rapid in the OP-1 treated group and was significantly lower than in the Nx controls (Figure 5: $p < 0.02$ at weeks 6 and 8; $p < 0.01$ at weeks 7 and 9). Similar results were observed in serum BUN values as well.

More important, measurements of GFR, based on serum and urine creatinine values, showed a highly significant decrease in both groups of nephrectomized rats (< 1.8 ml/min) relative to sham-operated controls (4.7 ± 1.1 ml/min). The GFR in both groups continued to decline during weeks 1-3 of treatment. At approximately three weeks, however, GFR in the OP-1 treated group stabilized whereas the decline in renal function continued in the Nx controls. By week 5, the difference in GFR values between OP-1 treated and Nx control rats had become statistically significant ($p < 0.02$). This difference in GFR continued to increase over time ($p < 0.01$ at week 6; $p < 0.001$ at weeks 7 and 8), as the Nx controls continued to decline but the OP-1 treated rats remained stable (Figure 6). By the end of 9 weeks, 40% of the Nx control rats were dead whereas none of the OP-1 treated rats had died.

Histological evaluation of tissue sections confirmed that OP-1 treated rats showed greater preservation or maintenance of glomeruli, as well as proximal and distal tubule structures. There were also signs in the OP-1 treated rats of nephrogenic mesenchymal condensations and the appearance of developmental nephrogenic structures. Table 1 reports results of several standard quantitative (e.g., PAS-staining of extracellular matrix) and semi-quantitative (e.g., visual ranking) histomorphometric measures obtained for tissue slices from Nx control and OP-1 treated Nx rats. These results indicate that OP-1 treatment of nephrectomized rates resulted in overall improvement (or reduced degeneration) of kidney tissue morphology, increased mesangial or perivascular thickening,

decreased glomerular sclerosis and loop collapse, decreased presence of "scattered" sclerosis and microaneurysms, and an increase in viable glomeruli.

TABLE 1

Group	Normal Histology	Mesangial Thickening	Glomerular Sclerosis & Loop Collapse	Scattered Sclerosis & Microaneurysms	Absence of Viable Glomeruli
Control (N=15)	2.58 ±0.22	27.3 ±2.4	26.5±3.5	34.7±4.2	8.9±0.7
OP-1 (N=20)	11.41±1.1	58.6±3.2	14.7±1.3	11.8±1.1	2.5±0.2
Significance	p <0.01	p <0.01	p <0.02	p <0.01	p <0.01

Equivalents

- 5 The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes which
- 10 come within the meaning and range of equivalency of the claims are intended to be embraced therein.

CLAIMS

What is claimed is:

- 1 1. A method of treatment for a mammal in, or at risk of, chronic renal failure
2 comprising
3 administering to said mammal a therapeutically effective amount of an
4 OP/BMP renal therapeutic agent.
- 1 2. A method of treatment to delay the need for, or reduce the frequency of,
2 chronic dialysis treatments comprising
3 administering to a mammal a therapeutically effective amount of an
4 OP/BMP renal therapeutic agent.
- 1 3. A method as in claim 1 wherein said renal therapeutic agent comprises a
2 polypeptide consisting of at least a C-terminal cysteine domain of a protein
3 selected from the group consisting of a pro form, a mature form, and a soluble
4 form of a polypeptide selected from the group consisting of OP-1, OP-2, OP-3,
5 BMP2, BMP3, BMP4, BMP5, BMP6, and BMP9.
- 1 4. A method as in claim 3 wherein said renal therapeutic agent comprises a
2 polypeptide consisting of at least a C-terminal cysteine domain of a protein
3 selected from the group consisting of a pro form, a mature form, and a soluble
4 form of human OP-1.
- 1 5. A method as in claim 1 wherein said renal therapeutic agent comprises a
2 polypeptide having at least 70% homology with an amino acid sequence of a C-
3 terminal seven-cysteine domain of human OP-1.
- 1 6. A method as in claim 5 wherein said polypeptide has at least 75%
2 homology with an amino acid sequence of a C-terminal seven-cysteine domain of
3 human OP-1.

1 7. A method as in claim 5 wherein said polypeptide has at least 80%
2 homology with an amino acid sequence of a C-terminal seven-cysteine domain of
3 human OP-1.

1 8. A method as in claim 5 wherein said polypeptide has at least 60% identity
2 with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-
3 1.

1 9. A method as in claim 5 wherein said polypeptide has at least 65% identity
2 with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-
3 1.

1 10. A method as in claim 5 wherein said polypeptide has at least 70% identity
2 with an amino acid sequence of a C-terminal seven-cysteine domain of human OP-
3 1.

1 11. A method as in any one of claims 3-10 wherein said renal therapeutic agent
2 (a) induces chondrogenesis in an ectopic bone assay;
3 (b) prevents, inhibits, delays or alleviates loss of renal function in an animal
4 model of chronic renal failure; or
5 (c) causes a clinically significant improvement in a standard marker of renal
6 function when administered to a mammal in, or at risk of, chronic renal failure.

1 12. A method as in claim 1 wherein said renal therapeutic agent is selected
2 from the group consisting of human osteogenic proteins and human bone
3 morphogenetic proteins.

1 13. A method as in any one of claims 1-12 wherein
2 said mammal is afflicted with a condition selected from the group
3 consisting of chronic renal failure, end-stage renal disease, chronic diabetic
4 nephropathy, diabetic glomerulopathy, diabetic renal hypertrophy, hypertensive

5 nephrosclerosis, hypertensive glomerulosclerosis, chronic glomerulonephritis,
6 hereditary nephritis, and renal dysplasia.

1 14. A method as in any one of claims 1-12 wherein
2 examination of a renal biopsy of said mammal indicates that said mammal is
3 afflicted with a condition selected from the group consisting of glomerular
4 hypertrophy, tubular hypertrophy, glomerulosclerosis, and tubulointerstitial
5 sclerosis.

1 15. A method as in any one of claims 1-12 wherein
2 examination of said mammal indicates renal fibrosis.

1 16. A method as in claim 15 wherein
2 said examination is an ultrasound, MRI or CAT scan of said mammal.

1 17. A method as in any one of claims 1-12 wherein
2 said mammal possesses a number of functional nephron units which is less
3 than about 50% of a number of functional nephron units present in a mammal
4 having intact healthy kidneys.

1 18. A method as in any one of claims 1-12 wherein
2 said mammal possesses a number of functional nephron units which is less
3 than about 40% of a number of functional nephron units present in a mammal
4 having intact healthy kidneys.

1 19. A method as in any one of claims 1-12 wherein
2 said mammal possesses a number of functional nephron units which is less
3 than about 30% of a number of functional nephron units present in a mammal
4 having intact healthy kidneys.

1 20. A method as in any one of claims 1-12 wherein

2 said mammal possesses a number of functional nephron units which is less
3 than about 20% of a number of functional nephron units present in a mammal
4 having intact healthy kidneys.

1 21. A method as in any one of claims 1-12 wherein
2 said mammal is a kidney transplant recipient.

1 22. A method as in any one of claims 1-12 wherein
2 said mammal possesses only one kidney.

1 23. A method as in any one of claims 1-12 wherein
2 examination of a urinary sediment of said mammal indicates a presence of
3 broad casts.

1 24. A method as in any one of claims 1-12 wherein
2 said mammal has a GFR which is chronically less than about 50% of a
3 GFR_{exp} for said mammal.

1 25. A method as in claim 24 wherein
2 said mammal has a GFR which is chronically less than about 40% of a
3 GFR_{exp} for said mammal.

1 26. A method as in claim 24 wherein
2 said mammal has a GFR which is chronically less than about 30% of a
3 GFR_{exp} for said mammal.

1 27. A method as in claim 24 wherein
2 said mammal has a GFR which is chronically less than about 20% of a
3 GFR_{exp} for said mammal.

1 28. A method as in any one of claims 1-12 wherein
2 said mammal is a human male weighing at least about 50 kg and has a GFR
3 which is chronically less than about 50 ml/min.

- 1 29. A method as in claim 28 wherein
2 said mammal is a human male weighing at least about 50 kg and has a GFR
3 which is chronically less than about 40 ml/min.
- 1 30. A method as in claim 28 wherein
2 said mammal is a human male weighing at least about 50 kg and has a GFR
3 which is chronically less than about 30 ml/min.
- 1 31. A method as in claim 28 wherein
2 said mammal is a human male weighing at least about 50 kg and has a GFR
3 which is chronically less than about 20 ml/min.
- 1 32. A method as in any one of claims 1-12 wherein
2 said mammal is a human female weighing at least about 40 kg and has a
3 GFR which is chronically less than about 40 ml/min.
- 1 33. A method as in claim 32 wherein
2 said mammal is a human female weighing at least about 40 kg and has a
3 GFR which is chronically less than about 30 ml/min.
- 1 34. A method as in claim 32 wherein
2 said mammal is a human female weighing at least about 40 kg and has a
3 GFR which is chronically less than about 20 ml/min.
- 1 35. A method as in claim 32 wherein
2 said mammal is a human female weighing at least about 40 kg and has a
3 GFR which is chronically less than about 10 ml/min.
- 1 36. A method as in any one of claims 1-12 wherein said treatment reduces
2 serum creatinine levels in said mammal by at least about 5% over 3 months.
- 1 37. A method as in any one of claims 1-12 wherein

2 prior to said treatment said mammal presented a chronic decline in a
3 clinical indicator of renal function; and

4 after at least about 3 months of said treatment, said indicator stabilizes.

1 38. A method as in any one of claims 1-12 wherein said administration is oral.

1 39. A method as in any one of claims 1-12 wherein said administration is
2 parenteral.

1 40. A method as in claim 39 wherein said administration is intravenous.

1 41. A method as in claim 39 wherein said administration is intraperitoneal.

1 42. A method as in claim 39 wherein said administration is into the renal
2 capsule.

1 43. A method as in claim 39 wherein a stent has been implanted into said
2 mammal for said administration.

1 44. A method as in claim 43 wherein said stent is an intravenous stent.

1 45. A method as in claim 43 wherein said stent is an intraperitoneal stent.

1 46. A method as in claim 43 wherein said stent is a renal intracapsular stent.

1 47. A method as in claim 39 wherein said administration is by an implanted
2 device.

1 48. A method as in any one of claims 1-12 wherein said administration is at
2 least once a week for a period of at least about one month.

1 49. A method as in any one of claims 1-12 wherein said administration is at
2 least once a month for a period of at least about one year.

1 50. A method as in any one of claims 1-12 wherein said renal therapeutic agent
2 is administered at a dosage of about 0.01-1200 $\mu\text{g/kg}$ body weight of said
3 mammal.

1 51. A method as in claim 50 wherein said renal therapeutic agent is
2 administered at a dosage of about 10-300 $\mu\text{g/kg}$ body weight of said mammal.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093
1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104
1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117
1118
1119
1120
1121
1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180
1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416
1417
1418
1419
1420
1421
1422
1423
1424
1425
1426
1427
1428
1429
1430
1431
1432
1433
1434
1435
1436
1437
1438
1439
1440
1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534
1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
1594
1595
1596
1597
1598
1599
1600
1601
1602
1603
1604
1605
1606
1607
1608
1609
1610
1611
1612
1613
1614
1615
1616
1617
1618
1619
1620
1621
1622
1623
1624
1625
1626
1627
1628
1629
1630
1631
1632
1633
1634
1635
1636
1637
1638
1639
1640
1641
1642
1643
1644
1645
1646
1647
1648
1649
1650
1651
1652
1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711
1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865
1866
1867
1868
1869
1870
1871
1872
1873
1874
1875
1876
1877
1878
1879
1880
1881
1882
1883
1884
1885
1886
1887
1888
1889
1890
1891
1892
1893
1894
1895
1896
1897
1898
1899
1900
1901
1902
1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947
1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
2028
2029
2030
2031
2032
2033
2034
2035
2036
2037
2038
2039
2040
2041
2042
2043
2044
2045
2046
2047
2048
2049
2050
2051
2052
2053
2054
2055
2056
2057
2058
2059
2060
2061
2062
2063
2064
2065
2066
2067
2068
2069
2070
2071
2072
2073
2074
2075
2076
2077
2078
2079
2080
2081
2082
2083
2084
2085
2086
2087
2088
2089
2090
2091
2092
2093
2094
2095
2096
2097
2098
2099
2100
2101
2102
2103
2104
2105
2106
2107
2108
2109
2110
2111
2112
2113
2114
2115
2116
2117
2118
2119
2120
2121
2122
2123
2124
2125
2126
2127
2128
2129
2130
2131
2132
2133
2134
2135
2136
2137
2138
2139
2140
2141
2142
2143
2144
2145
2146
2147
2148
2149
2150
2151
2152
2153
2154
2155
2156
2157
2158
2159
2160
2161
2162
2163
2164
2165
2166
2167
2168
2169
2170
2171
2172
2173
2174
2175
2176
2177
2178
2179
2180
2181
2182
2183
2184
2185
2186
2187
2188
2189
2190
2191
2192
2193
2194
2195
2196
2197
2198
2199
2200
2201
2202
2203
2204
2205
2206
2207
220

NOVEL THERAPIES FOR
CHRONIC RENAL FAILURE

Abstract of the Disclosure

The present invention provides methods for the treatment, and
pharmaceuticals for use in the treatment, of mammalian subjects in, or at risk of
chronic renal failure, or at risk of a need for renal replacement therapy. The
methods involve the administration of certain proteins of, or based upon, the
5 osteogenic protein/bone morphogenetic protein (OP/BMP) family within the TGF-
 β superfamily of proteins.

10

15

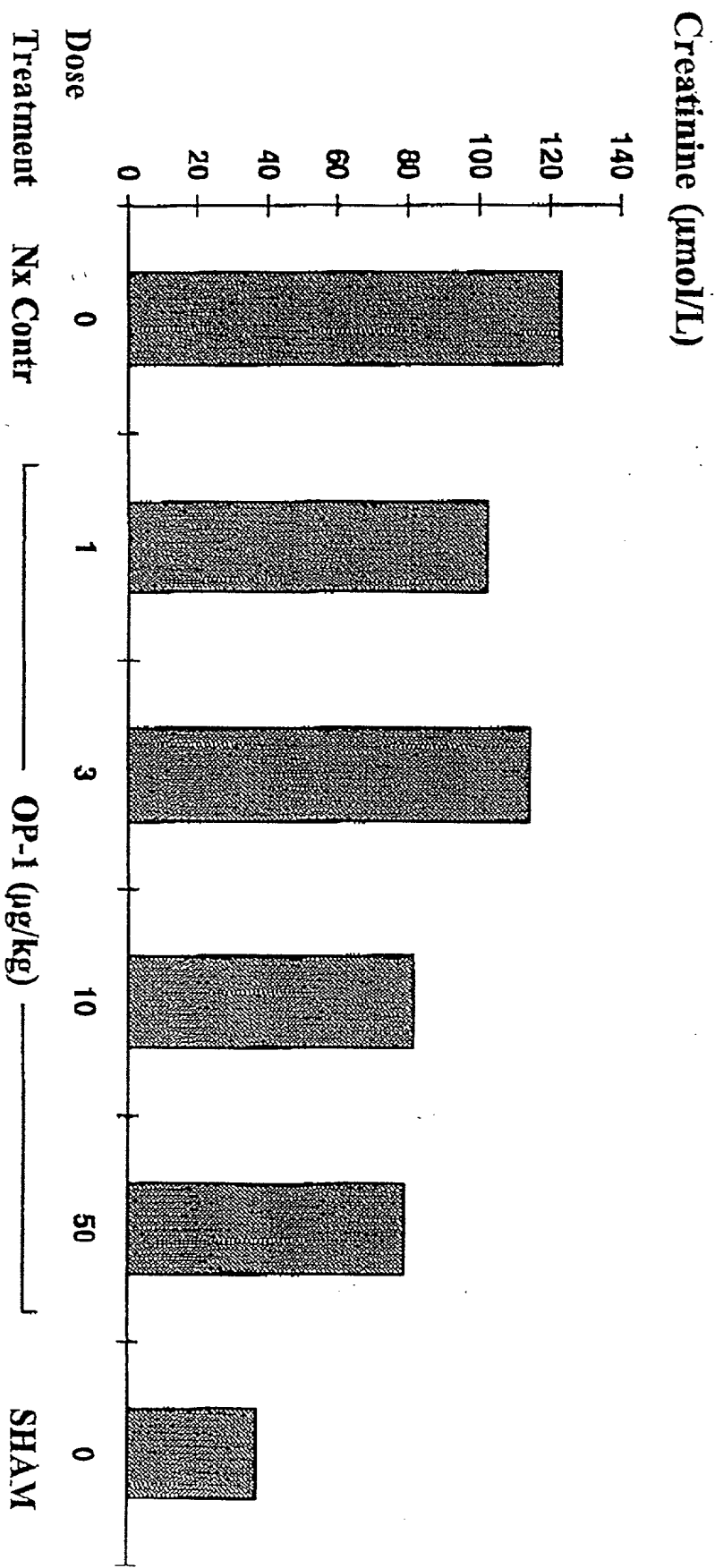


Figure 1

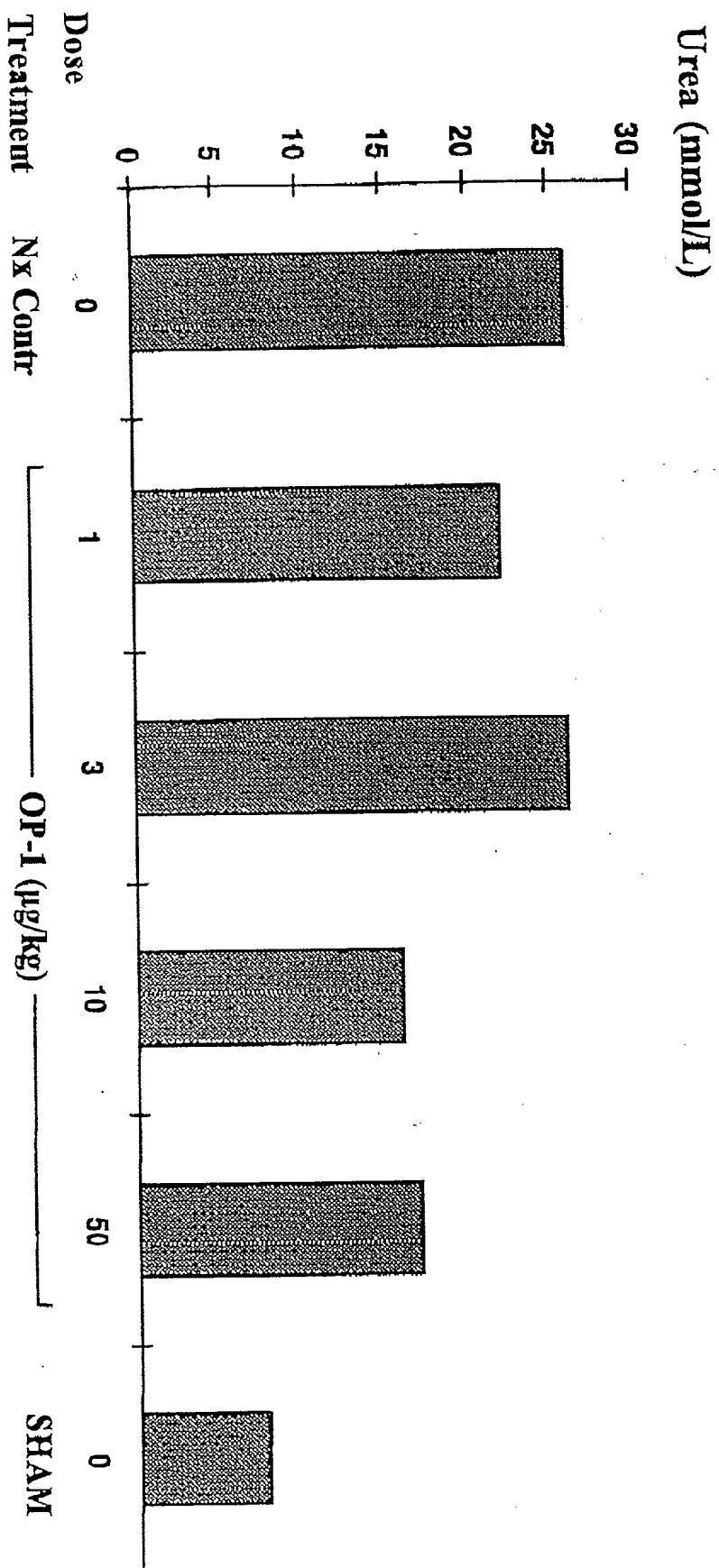
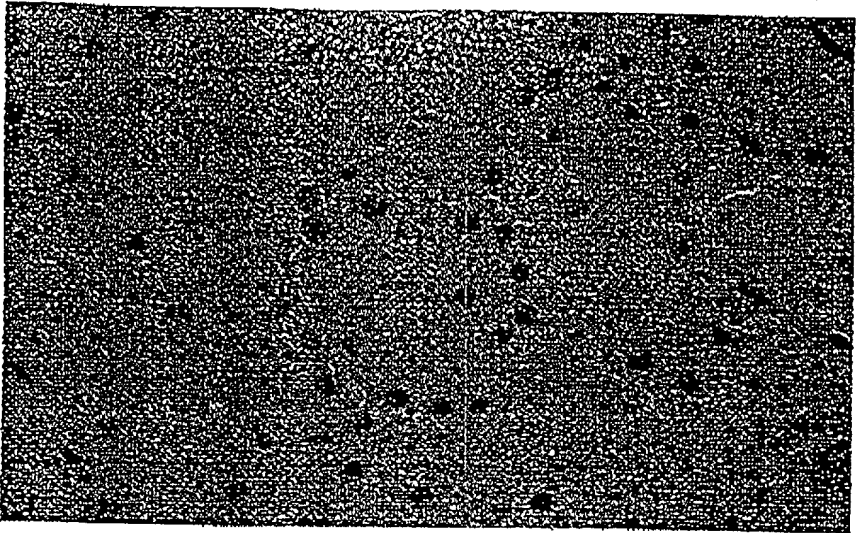
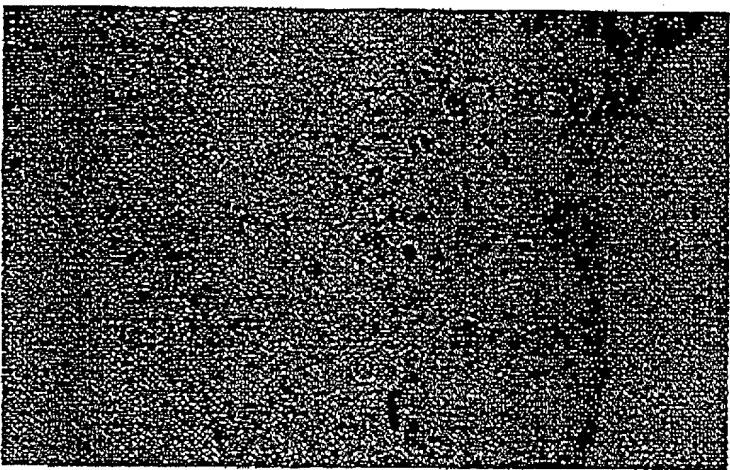


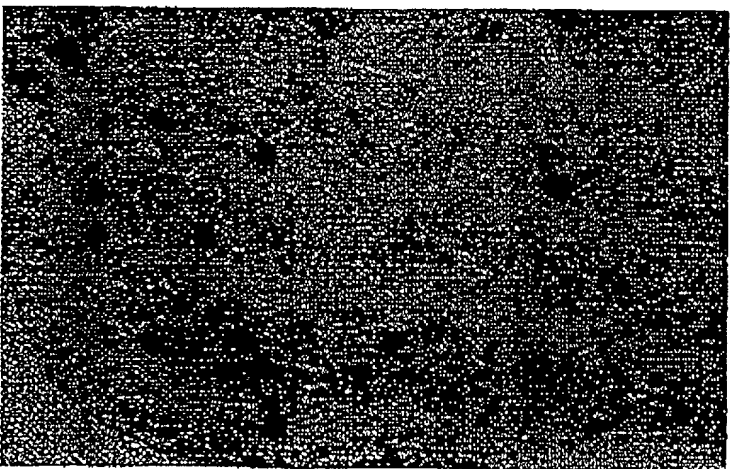
Figure 2



A



B



C

Figure 3

Figure 3 shows three micrographs (A, B, and C) of a surface covered with small, dark, circular or oval structures, possibly cells or particles. The structures are distributed across the surface, with some appearing more densely packed than others. The background is dark and grainy.

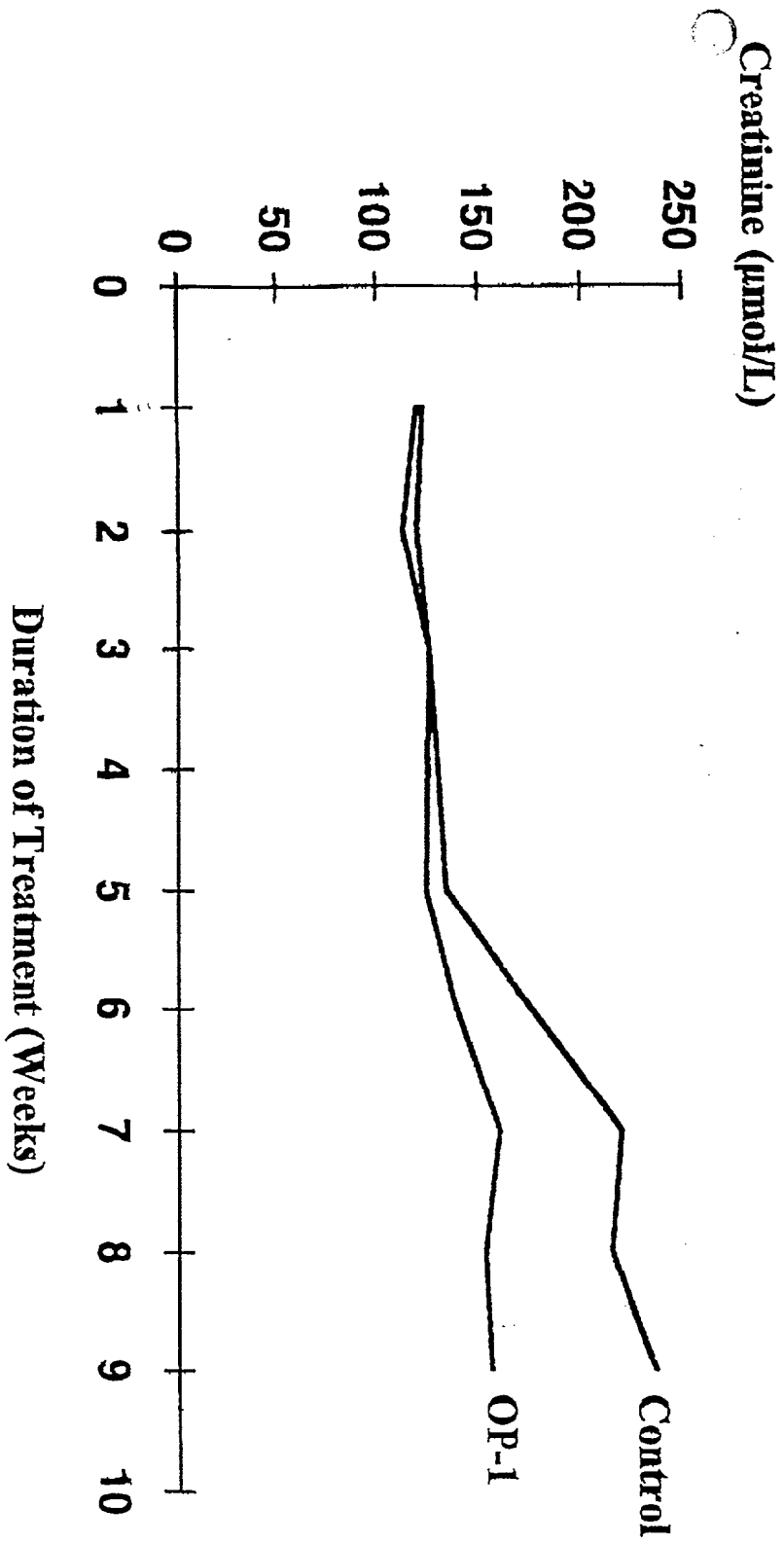


Figure 5

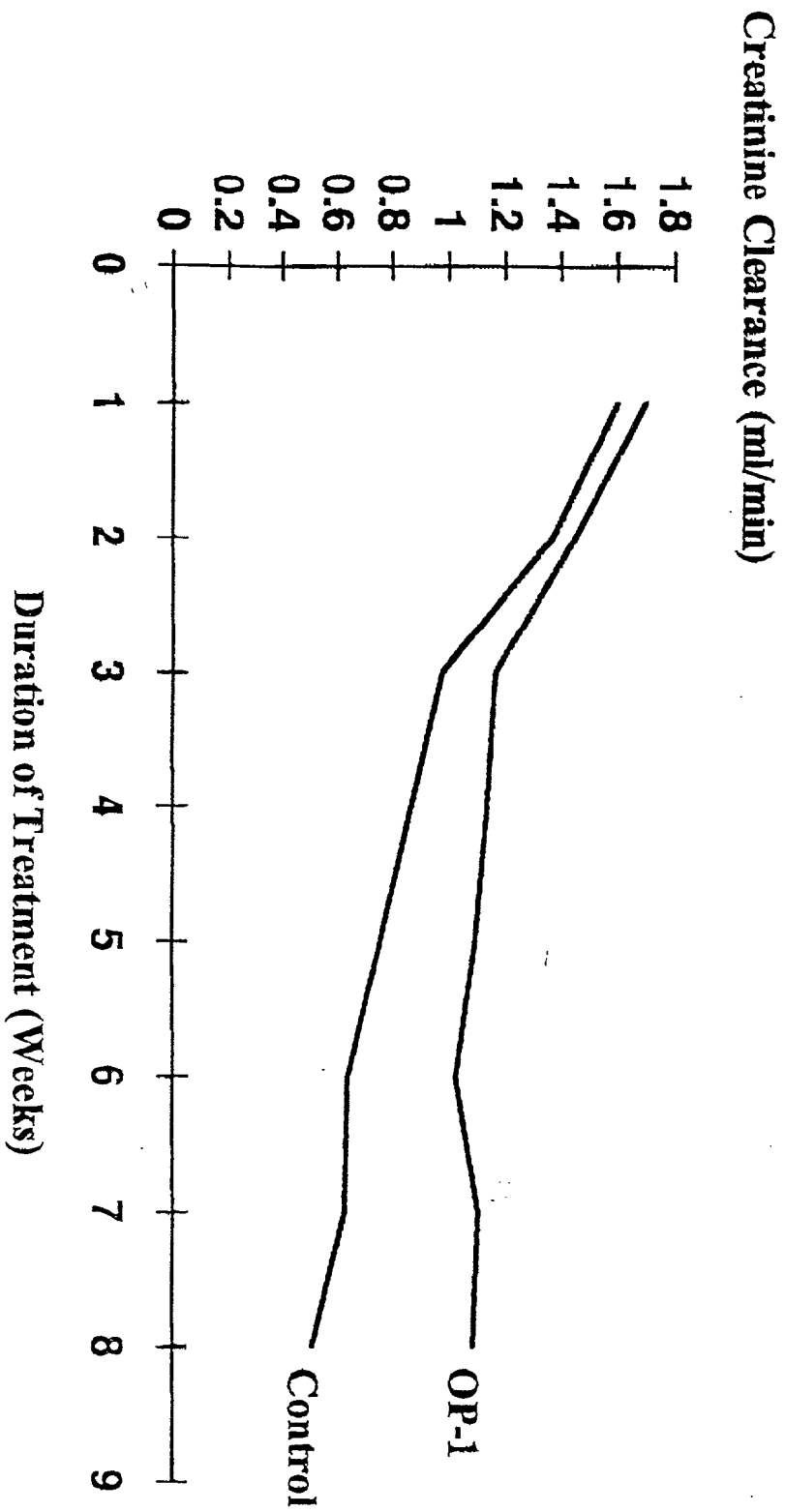


Figure 6

PATENT

Atty. Docket No. CRP-145

**COMBINED DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION**

(Original, Design, National Stage of PCT, Supplemental, Divisional, Continuation or CIP)

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NOVEL THERAPIES FOR CHRONIC RENAL FAILURE

the specification of which (check one):

- ☒ is attached hereto.
- ☐ was filed on _____ as Application Serial No. 0 / _____ or
- ☐ was described and claimed in PCT International Application No. _____ filed on _____ and as amended under PCT Article 19 on _____ (if any).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims as amended by any amendment referred to herein.

I acknowledge the continuing duty to disclose information which is material to the examination of this application in accordance with 37 C.F.R. §1.56.

PRIORITY CLAIM

- ☐ A. I hereby claim benefit under 35 U.S.C. 119(e) of United States Provisional Application No. _____, filed on _____.
- ☐ B. I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and I have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

- ☐ no such applications have been filed.
- ☐ such applications have been filed as follows:

**EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN
12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO
THIS U.S. APPLICATION**

Country	Application Number	Date of Filing (mo., day, year)	Priority Claimed Under 35 USC 119
			<input type="checkbox"/> YES NO <input type="checkbox"/>
			<input type="checkbox"/> YES NO <input type="checkbox"/>
			<input type="checkbox"/> YES NO <input type="checkbox"/>

- ☐ C. I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose material information as defined in 37 C.F.R. §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

**PRIOR U.S. NON-PROVISIONAL APPLICATIONS OR PCT INTERNATIONAL
APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 USC §120:**

U.S. APPLICATIONS	U.S. FILING DATE	STATUS
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, aband.)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, aband.)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, aband.)

POWER OF ATTORNEY

As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the United States Patent and Trademark Office connected therewith:

Steven M. Bauer	Reg. No. 31,481
Paula A. Campbell	Reg. No. 32,503
Joseph A. Capraro, Jr.	Reg. No. 36,471
John J. Cotter	Reg. No. 38,116
Gillian M. Fenton	Reg. No. 36,508
Duncan A. Greenhalgh	Reg. No. 38,678
Robin D. Kelley	Reg. No. 34,637
Douglas J. Kline	Reg. No. 35,574
John D. Lanza	Reg. No. 40,060
Robin R. Longo	Reg. No. 40,071
Thomas C. Meyers	Reg. No. 36,989
Edmund R. Pitcher	Reg. No. 27,829
Kurt Rauschenbach	Reg. No. 40,137
Gretchen A. Rice	Reg. No. 37,429
J. Scott Southworth	Reg. No. 39,382
Christopher W. Stamos	Reg. No. 35,370
Robert J. Tosti	Reg. No. 35,393
Thomas A. Turano	Reg. No. 35,722
Michael J. Twomey	Reg. No. 38,349
Christine C. Vito	Reg. No. 39,061

Direct correspondence to:

Patent Administrator
Testa, Hurwitz & Thibault, LLP
High Street Tower
125 High Street
Boston, MA 02110

Direct telephone calls to:

Michael J. Twomey (617) 248-7362

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE(S)

Charles M. Cohen, Ph.D.

Full name of inventor

USA

Citizenship

Inventor's signature

Date

1 Harrington Lane, Weston, MA 02139

Residence

same

Post Office Address

Kuber T. Sampath, Ph.D.

Full name of inventor

USA

Citizenship

Inventor's signature

Date

98 Pamela Drive, Holliston, MA 01746

Residence

same

Post Office Address